**Remarks**

## Introduction

The claims have been amended. Claims 23 and 25-27 have been amended to more clearly set forth the subject matter of the invention. New Claim 28 has been added. Claims 7-19 have been cancelled, without prejudice. Applicants reserve the right to pursue the subject matter of claims 7-19 in a divisional application. Claims 27, 5-6, 20-26, and 28 are currently pending. Claim 27 is the sole independent claim.

## Section 112 rejections

**Claims 23-25**

Claims 23-25 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. Specifically, the Examiner alleges that "one of skill in the art is unable to fully predict possible results from the administration of the compound of claim 27 due to the unpredictability of the role of the inhibition of cellular levels of amyloid β, and since the treatment of Alzheimer's disease is mediated by the breakdown of acetylcholine or the inhibition of excess amounts of glutamate." Applicants respectfully submit that the amendments to claims 23 and 25 obviate these grounds of rejection.

As the Examiner has pointed out, there are only two types of drugs which the United States Food and Drug Administration (FDA) has approved for treatment of Alzheimer's Disease. Both are directed toward neurotransmitter deficiencies. The first type (including Aricept, Exelon, Reminyl, and Cognex) delays the breakdown of acetylcholine, and the second type (Memantine) is directed toward inhibition of glutamate which can damage nerve cells.

However, FDA approval is not alone determinative of whether a particular therapy is an effective treatment of a disease. The delay in FDA approval of Memantine is a good example. Memantine was available for treatment of Alzheimer's in Germany for two decades prior to FDA approval. This does not reflect on the ability of the drug's performance as an effective treatment of Alzheimer's, but on the other hand, is merely reflective of a delay in satisfying the FDA's criteria to demonstrate the drug's safety and efficacy.

The current state of the art clearly demonstrates that accumulation of amyloid β protein in the brain is a cause of Alzheimer's Disease, as well as other diseases. Several medical journal articles and United States Patents have been attached to this response, which support this. As is shown below, amyloid β protein deposits contribute to many similar conditions attributed to the production of amyloid β, including amyloid angiopathy, cerebral amyloid angiopathy, systemic amyloidosis, hereditary cerebral hemorrhage with amyloidosis of the Dutch type, inclusion body myositis, and Down's syndrome.

U.S. Patent No. 6,670,182, column 1, lines 48-55:

"A characteristic feature of Alzheimer's disease is the formation or deposit of β-amyloid plaques in affected individuals. Mature β-amyloid plaques are often associated with degenerating neuronal processes. β-amyloid deposits are not solely associated with persons suffering from Alzheimer's disease but are also associated with persons suffering from other amyloidoses, for example, brain trauma or Downs syndrome."

U.S. Patent No. 6,607,758, column 1, lines 27-29:

"Accumulating evidence implicates amyloid as a major causative factor of Alzheimer's disease pathogenesis."

Column 1, lines 51-55:

"The amyloid diseases include, but are not limited to, the amyloid associated with Alzheimer's disease, Down's syndrome and hereditary cerebral hemorrhage with amyloidosis of the Dutch type."

U.S. Patent No. 6,673,600, column 2, lines 1-2:

"There is a large amount of evidence that Aβ peptide is a crucial factor in the development of Alzheimer's disease."

Berger, Abi. "Amyloid Clearly Implicated in Alzheimer's Disease" *British Medical Journal,* July 11, 1998:

"Dr. Geula and colleagues have now shown that not only is B amyloid present in Alzheimer's disease, but it is almost definitely causative."

Conova, Susan. "Is Alzheimer's an Astrocyte Disease?" *In Vivo*, Columbia University Health Sciences, Vol. 2, No. 6, March 26, 2003:

"Alzheimer's, most researchers believe, is caused when a small peptide, beta-amyloid, accumulates in the brain."

After considering the abundance of research demonstrating that amyloid β is a cause of Alzheimer's, it logically follows that to reduce or prevent amyloid β accumulation in the brain will prevent or improve the condition of one suffering from or at risk for developing Alzheimer's or another amyloid β related disease. In fact, several U.S. patents claim methods of treating Alzheimer's by inhibiting amyloid β. These include U.S. Patent No. 6,469,055, claim 2; U.S. Patent No. 6,311,408, claim 54; and U.S. Patent No. 6,607,758; claim 8.

All of the above listed U.S. patents conducted *in vitro* assays to demonstrate activity inhibiting amyloid β. Conducting *in vitro* assays are well-known and accepted in the art as a method of testing the activity of a particular compound. If a particular compound demonstrates a desired activity in an *in vitro* assay, it is indicative that the compound will possess the same activity *in vivo*. By demonstrating the activity by *in vitro* assay, the Applicants in each of the above-referenced patents were able to satisfy the enablement requirement of Section 112, first paragraph.

Similarly, in the present invention, *in vitro* assays were conducted to show the activity of the compounds of the present invention. As shown in example 636, the compounds of the present invention were tested for and demonstrated the ability to inhibit amyloid beta production.

The state of the art reflects that amyloid β is a causative factor in Alzheimer's disease and that preventing or removing amyloid β plaques would provide a treatment for those afflicted with

the disease, or similar amyloid β related diseases. Therefore, Applicants submit that Claims 23-25 meet the enablement requirement of Section 112, first paragraph. Reconsideration and withdrawal of the rejections of claims 23-25 are therefore appropriate and respectfully requested.

**Claims 21-22**

Claims 21-22 have also been rejected under 35 U.S.C. §112, first paragraph, as not meeting the enablement requirement. Specifically, the Examiner states, "the specification, while being enabling for the inhibition of proteolytic cleavage of amyloid beta precursor protein does not reasonable provide enablement for the modulating of the level of amyloid beta precursor protein, either by increasing or decreasing." Applicants respectfully submit that the clarifying amendment of claim 21 obviates this rejection.

It is well-known in the art that by administering a compound that exhibits inhibitory activity of another compound, that the other compound will likely decrease upon said administration. Similarly, after administering said first compound, if the amount of the administration of the compound is decreased or ceased, an increase in the other compound will likely result. This is method of modulation.

The Examiner had acknowledged that the compounds of the present invention demonstrate inhibitory activity to proteolytic cleavage of amyloid beta precursor protein, which is the mechanism for the production of amyloid beta. This is pointed out in the specification at page 1, line 21. The method of modulation of amyloid beta by administering a compound of the present invention would then be within the means of one of skill in the art. Therefore, reconsideration and withdrawal of the rejections of claims 21-22 are therefore appropriate and respectfully requested.

**Claim 26**

Claim 26 has been rejected under 35 U.S.C. §112, first and second paragraphs as failing to comply with the written description requirement and as being indefinite, respectively.

Specifically, the Examiner states that the alleged "abbreviation," "Aβ" is not defined in the specification. Applicants respectfully submit that the amendment of claim 26 obviates this ground of rejection by clarifying the meaning of "Aβ," which is synonymous with amyloid β.

"Aβ" is not an unsupported abbreviation as the Examiner suggests. The meaning of "Aβ" is readily ascertained from the context in which it is used in the application. In the paragraph that the Examiner points out at page 311, the terms "Aβ" and amyloid β are used interchangeably and both directed toward explanation of the assay to determine inhibition of production of amyloid beta.

Furthermore, "Aβ" is a well-known term used to describe the same protein as the term amyloid β. This is demonstrated by a number of references and U.S. patents. These references are found in U.S. Patent No. 6,673,600, column 1, line 26; U.S. Patent No. 6,331,408, column 1, line 53, and in the Merck Frosst abstract of *Cell 97* (1999): 395-406.

Considering that the term "Aβ" is well-known in the art and supported in the specification, Applicants submit that one of skill in the art would recognize that "Aβ" refers to the same protein as the term amyloid β. However, for the sake of consistency, claim 26 has been amended by replacing the term "Aβ" with "amyloid β." This amendment is clearly not a narrowing amendment. Therefore, reconsideration and withdrawal of the rejections of claim 26 are appropriate and respectfully requested.

**Section 102 rejections**

Claims 27, 5, and 6 have been rejected under 35 U.S.C. §102(b) as being anticipated by Linfield et al. Applicants respectfully submit that the amendment to claim 27 obviates this ground of rejection. Reconsideration and withdrawal of the rejections are, therefore, appropriate and respectfully requested.

No fee is believed due with this submission. However, please charge any additional fees to our Deposit Account No. 08-2461.

In view of the amendments and remarks set forth above, reconsideration and withdrawal of the rejections are appropriate and respectfully requested. Applicants submit the present claims are patentably distinct over the art and allowable in form. Early allowance is therefore solicited. The Examiner is encouraged to contact the undersigned attorney should there be any questions regarding this amendment.

Respectfully submitted,

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*in vivo / in vē´ vō /, occurring in a living organism.*

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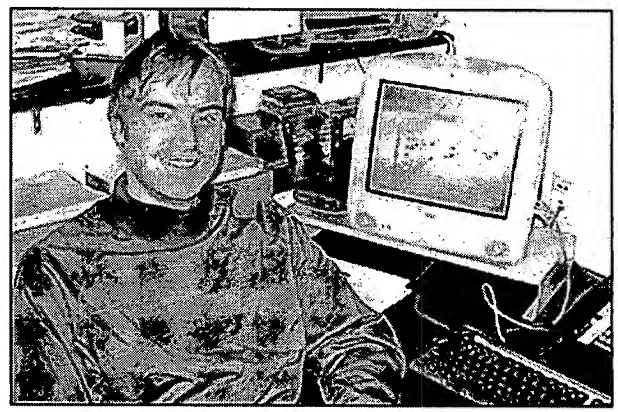
ALZHEIMER'S DISEASE

# Is Alzheimer's an Astrocyte Disease?

*New research suggests brain cells called astrocytes may have a role*

BY SUSAN CONOVA



Jens Husemann

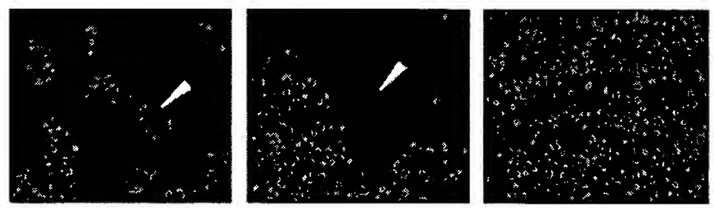
A new study from P&S and Stanford University suggests that brain cells called astrocytes may be behind the accumulation of beta-amyloid peptide in the brain that causes Alzheimer's disease.

Alzheimer's, most researchers believe, is caused when a small peptide, beta-amyloid, accumulates in the brain. The peptide is made throughout life, but in people with Alzheimer's, either too much is made or too little is degraded—or both—and the excess peptide forms aggregates that accumulate throughout the brain. The aggregated beta-amyloid has been implicated in neuron death, which eventually leads to dementia.

Researchers have known that cells called microglia, which surround beta-amyloid

deposits in the AD brain, can ingest and destroy the beta-amyloid peptide in cell culture, so they've been trying to stimulate the cells to do the same in vivo. But the role of astrocytes, which are also present at sites of beta-amyloid deposition, hadn't been examined.

The new findings show that normal adult astrocytes also can degrade beta-amyloid, suggesting that treatments to boost astrocyte activity in Alzheimer's may be beneficial. "This is the first study to show that adult astrocytes can degrade beta-amyloid," says Dr. Jens Husemann, the study's senior author and associate research scientist in the Department of Physiology and Cellular Biophysics. "In addition, astrocytes outnumber microglia in the brain, so they may be very important. Now labs will explore ways to activate astrocytes to increase beta-amyloid removal." The research was published March 3 on the Nature Medicine Web site and will appear in the April print issue.



*Adult astrocytes in the left figure (nuclei are stained blue) ingest and concentrate beta-amyloid around the nuclei. Within 24 hours, the cells, seen in the center photo, degrade the ingested proteins. The photo on the right shows beta-amyloid on a slide before astrocyte cells were added.*

The research group, including Dr. Samuel Silverstein, the John C. Dalton Professor of Physiology and Cellular Biophysics; Dr. John Loike, research scientist; and Dr. Tony Wyss-Coray from Stanford, also speculates that Alzheimer's may be caused by astrocyte dysfunction. It is still unknown why beta-amyloid accumulates in people with the late onset form of the disease, but one possibility is that the astrocytes fail to degrade the peptide. Dr. Husemann and his colleagues are now looking at astrocytes from the brains of Alzheimer's patients and mice with a similar Alzheimer's-like disease to see if their cells are still capable of destroying beta-amyloid.

In the Nature Medicine study, the researchers found that when they placed cultured adult mouse astrocytes onto brain tissue taken from Alzheimer's model mice, the astrocytes ingested beta-amyloid. The astrocytes reduced the amount of

beta-amyloid in the brain tissue by 40 percent within 24 hours.

To see if the astrocytes also degraded—as well as ingested—beta-amyloid, they incubated adult mouse astrocytes in media containing this peptide. At the beginning of the incubation, all beta-amyloid was in the media. In the next 24 hours, the researchers saw that all of the beta-amyloid moved into the astrocytes and then disappeared completely, indicating that the astrocytes had degraded the beta-amyloid and had not exported it.

Though it has been known that astrocytes can eat and destroy proteins in general, no one has published work showing that the cells could consume beta-amyloid specifically. Dr. Husemann says they only discovered the astrocyte's ability when they looked at adult cells instead of the more commonly studied neonatal cells. Only the adult cells from mice destroyed the beta-amyloid; the neonatal cells did not.

The difference between adult and neonatal cells raises a question about which is the right Alzheimer's research model. Neonatal cells are used more often since they are much easier to culture than adult cells, but Dr. Husemann says "we need to do more experiments to identify the differences between adult and neonatal astrocytes."

Though the researchers suggest improving the astrocytes' ability to degrade beta-amyloid may be therapeutic, they caution that other astrocyte functions may contribute to the disease. "Studies suggest that upon interaction with beta-amyloid astrocytes may release inflammatory molecules that damage neurons," Dr. Husemann says.

Last year, brain inflammation in patients halted the clinical trials conducted by a pharmaceutical company of an Alzheimer's vaccine. In mice, the vaccine was thought to reduce plaques and relieve disease symptoms by stimulating microglia to devour beta-amyloid deposits in the brain. "It will be a delicate balancing act to stimulate beta-amyloid removal while keeping inflammation down at the same time," Dr. Husemann says.

*The research was supported by the Alzheimer's Disease Research Center at P&S, the NIH, and the Alzheimer's Association.*

[Top]

# Amyloid clearly implicated in Alzheimer's disease.

**British Medical Journal**, July 11, 1998, by Abi Berger

The role of amyloid in Alzheimer's disease looks destined to rise from bit part to lead player with the publication of three papers in the July edition of Nature Medicine. Scientists from three centres have found new evidence that dearly implicates amyloid in the pathogenesis of Alzheimer's disease, as well as having devised a diagnostic test that seems to be fairly specific and created a potential new treatment for the disease.

One of the reasons that the precise role of fibrillar B amyloid has remained elusive, despite its undeniable presence in the brains of people with Alzheimer's disease, is that none of the experimental mouse models of the disease has exhibited the full range of pathological features found in human Alzheimer's disease. Transgenic mice that express amyloid precursor protein in the cerebral cortex do not seem to sustain neurological pathology, and little neuronal death occurs when rats are injected with quantities of amyloid similar to that found in an amyloid plaque. These findings seemed to show that amyloid is an important factor in Alzheimer's disease, but not the causative agent.

Changiz Geula and his colleagues at Harvard Medical School now suggest that one reason for these observations could be that Alzheimer's disease "may be specifically a primate disease, and that possibly we've been looking in the wrong place." By developing a primate model of Alzheimer's disease, Dr Geula and colleagues have now shown that not only is B amyloid present in Alzheimer's disease, but it is almost definitely causative (Nature Medicine 1998;4:827-32).

To test their hypothesis, Dr Geula and his team injected very tiny quantities of fibrillar B amyloid into the brains of rhesus monkeys. They were able to identify neuronal loss not only at the site of injection but also circumferentially around the centre of the injection. Injections of both soluble B amyloid and the solution used to carry the fibrillar form caused significantly smaller amounts of damage, demonstrating that it is the fibrillar B amyloid that kills neurones and not simply the act of injecting something into the brain.

In addition, Dr Geula's team observed that the injection of fibrillar B

amyloid seemed to induce an intense microglial reaction in a halo around the site of the injection, in the same pattern found in Alzheimer's disease, and also induced hyperphosphorylated tau, the protein that forms neurofibrillary tangles in the brains of people with Alzheimer's disease. Thus the three hallmarks of Alzheimer's disease were identified in the rhesus monkey model, and furthermore, young primate brains revealed little neuronal toxicity, while older primate brains proved much more susceptible to fibrillar amyloid. Alzheimer's disease therefore seems to be both species and age specific.

Claudio Soto and his colleagues at the New York University Medical Center have also been working with B amyloid. They have been studying the process of how normal amyloid peptides are induced to fold up into the abnormal conformation of amyloid fibrillar sheets and have been using this information to try to find a way to stop it happening. They have created synthetic "B sheet breaker peptides," which are similar in molecular structure to B amyloid peptides but which seem to be able to prevent the formation of amyloid sheets and amyloid deposition in animal models (Nature Medicine 1998;4:822-6). In addition, they have gone on to show that these peptides can degrade preformed amyloid fibrils in animals (in press). Both of these observations point to a potential therapeutic approach to Alzheimer's disease.

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**RELATED TERMS**

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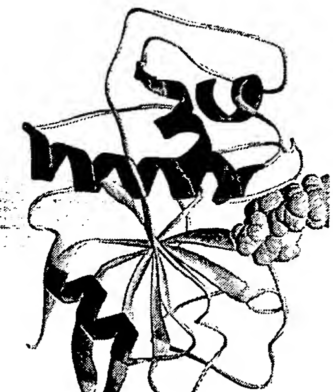
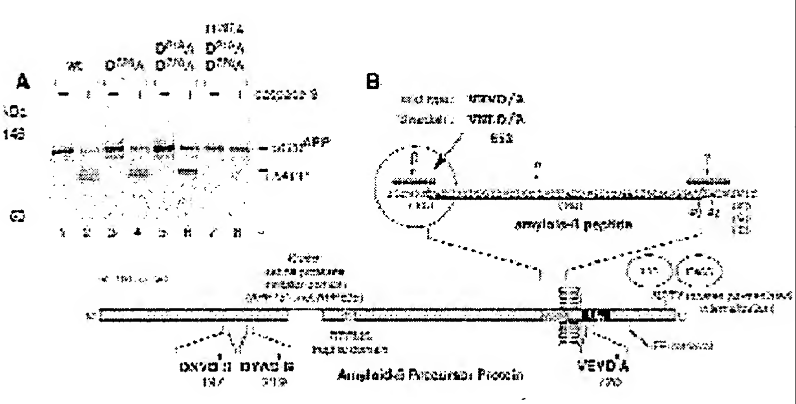


# Involvement of Caspases in Proteolytic Cleavage of Alzheimer's Amyloid-β Precursor Protein and Amyloidogenic Aβ Peptide Formation

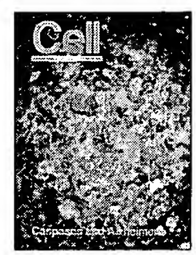
THE AMYLOID-β PRECURSOR PROTEIN (APP) is directly and efficiently cleaved by caspases during apoptosis, resulting in elevated amyloid-β(Aβ) peptide formation. The predominant site of caspase-mediated proteolysis is within the cytoplasmic tail of APP, and cleavage at this site occurs in hippocampal neurons *in vivo* following acute excitotoxic or ischemic brain injury. Caspase-3 is the predominant caspase involved in APP cleavage, consistent with its marked elevation in dying neurons of Alzheimer's disease brains and colocalization of its APP cleavage product with Aβ in senile plaques. Caspases thus appear to play a dual role in proteolytic processing of APP and the resulting propensity for Aβ peptide formation, as well as in the ultimate apoptotic death of neurons in Alzheimer's disease.




**Sites of caspase-mediated APP proteolysis. A. Identification and mutagenic elimination of sites of caspase-mediated proteolysis of amyloid-J precursor protein. B. Location of caspase cleavage sites within the amyloid-J precursor protein in relation to other structural features.**

**Citation • Abstract of:**

*Cell 97 (1999): 395-406.*

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